GEL ELECTROPHORESIS[4]

OBJECTIVES:

1. Understand the biotechnological importance and mechanism of agarose gel electrophoresis.
2. Practice using gel electrophoresis to stain and visualize DNA samples.
3. Use gel electrophoresis to determine the presence or absence of *Wolbachia* genetic products from prior labs, and the quantity and genetic length of the sample, if present.
4. Prepare positive samples for submission to Sanger sequencing.

LAB SAFETY REQUIREMENT: This lab requires you to wear a lab coat, safety glasses/goggles, and gloves to protect you from chemicals and to reduce the risk of contaminating samples with your DNA.

READ THIS LAB BEFORE COMING TO LAB SO YOU CAN ANSWER THE PRE-LAB QUESTIONS AND BRING THEM TO LAB

In this lab, you will use agarose gel electrophoresis to determine the presence and size of two different gene fragments amplified in the prior lab by PCR. The two genes you are amplifying are a mitochondrial gene (Cytochrome c oxidase I) found in all eukaryotes (including insects), and a version of a 16S rDNA found only in *Wolbachia*.

Introduction to gel electrophoresis

In our prior lab, you learned about the powerful biotechnology tool PCR and it's ability to amplify many copies of DNA in a short period of time. In this lab, you will learn about an equally powerful tool, **gel electrophoresis,** which is widely used to identify the quantity and size of DNA, proteins, and other kinds of molecules.

The principle of gel electrophoresis is simple (Fig. 1). Most chemicals naturally have chemical charges, and DNA is negatively charged. If placed within an electrical current, *DNA will be repelled away from the negative charge and be attracted to the positive charge*. In gel electrophoresis, the DNA is placed within a gel that has a structure like a sponge, with pores of various sizes. (The polysaccharide **agar** or **agarose** used to produce the gel is produced from a red algae seaweed, and is also the chemical that microbiologists used when culturing bacteria in Petri dishes.) An agarose gel is created by suspending dry agarose in a buffer solution, boiling until the solution becomes clear, and then pouring it into a casting tray and allowing it to cool. The result is a flexible gelatin-like slab.

Loading wells are oriented at the top of the gel to allow for precise insertion of PCR products into the gel. DNA migrates through the gel in a single, vertical **lane**. Several factors influence how fast

[4] Lab modified from Discover the Microbes Within: The *Wolbachia* Project. https://wolbachiaproject.org/

the DNA moves, including (i) the strength of the electrical field, (ii) the concentration of agarose, and, most importantly, (iii) the size of the DNA molecules. As the fragments of DNA move through the agarose gel lattice, smaller fragments will travel faster through the maze of holes while larger fragments travel slower. When the electrical current is stopped (and a chemical dye that binds to the DNA is added to the gel to visualize where the DNA travelled), the end result is that smaller fragments will have traveled farther distances than the larger ones. In other words, gel electrophoresis sorts chemical molecules by their size.

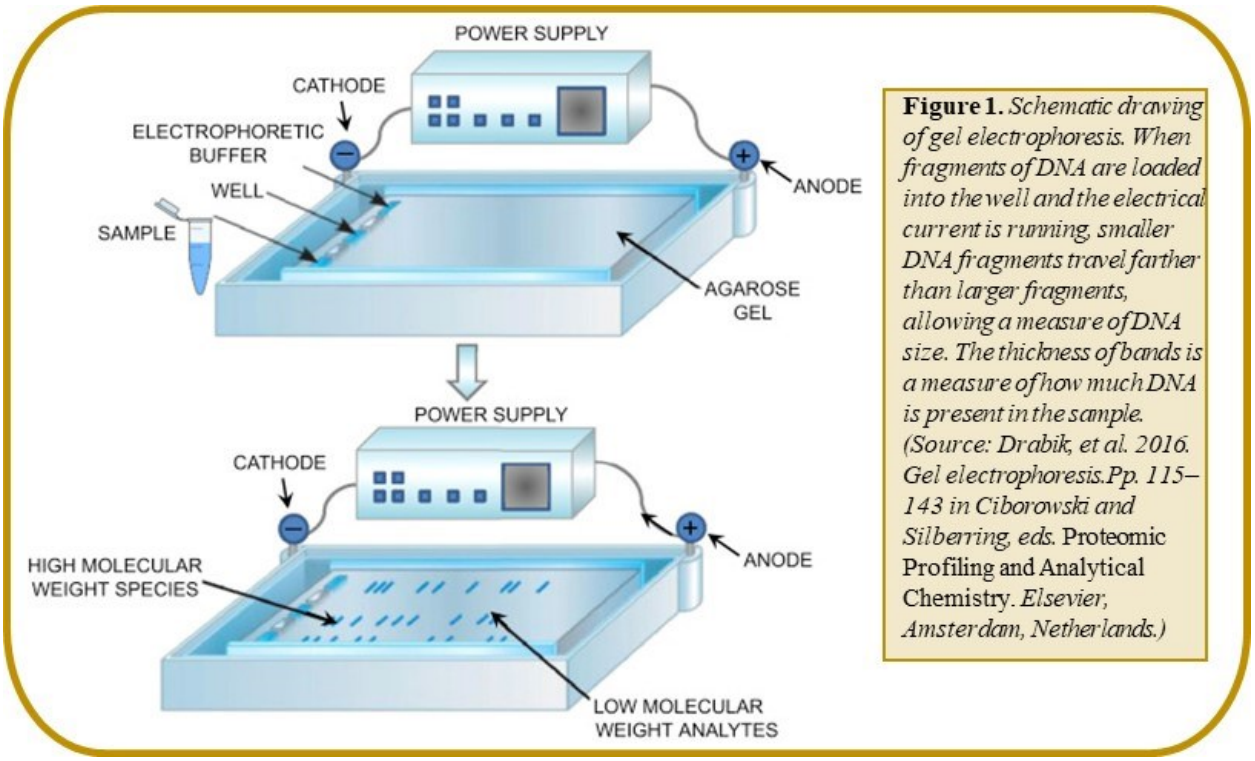


Figure 1. *Schematic drawing of gel electrophoresis. When fragments of DNA are loaded into the well and the electrical current is running, smaller DNA fragments travel farther than larger fragments, allowing a measure of DNA size. The thickness of bands is a measure of how much DNA is present in the sample. (Source: Drabik, et al. 2016. Gel electrophoresis. Pp. 115–143 in Ciborowski and Silberring, eds.* Proteomic Profiling and Analytical Chemistry. *Elsevier, Amsterdam, Netherlands.)*

DNA Visualization

DNA itself is not visible within an agarose gel. Therefore, a fluorescent stain is added to the gel that binds DNA and fluoresces under UV or blue light. DNA will appear as a horizontal line, or band, on the agarose gel (Fig. 2). Molecules of a similar size will travel in the same way, creating discrete bands that biologists can use to separate the chemical contents of their chemical mixtures. An additional benefit of gel electrophoresis is that the thickness of bands is also informative. If a sample has many molecules of the same size, the resulting band will be more visible. In other words, the "brightness" of a band is related to how much of a certain-sized molecule is present in the sample.

To assist in identifying the size is sample fragments, a **DNA ladder** is often added as a control to one of the wells. The ladder includes DNA fragments of known sizes. As electrophoresis occurs, the different fragments move at different speeds, creating reference bands of known DNA sizes. By comparing these bands of known sizes, it is straightforward to infer the size of the DNA in your sample.

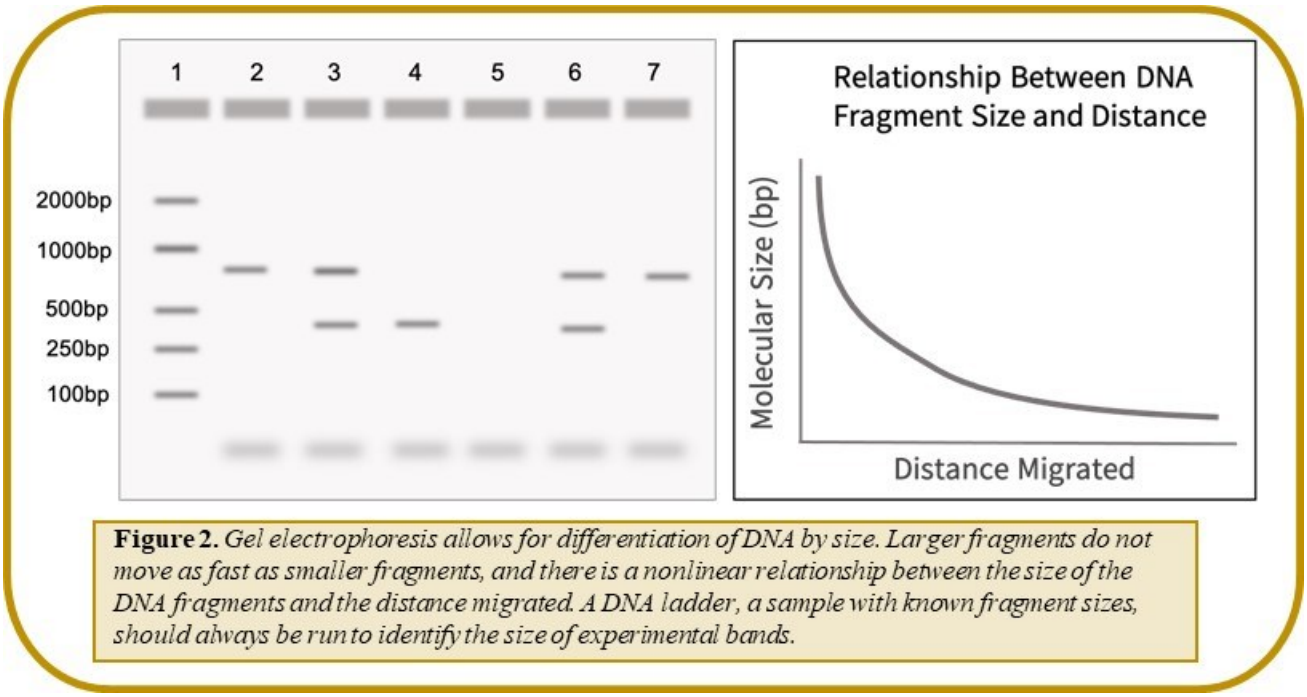


Figure 2. *Gel electrophoresis allows for differentiation of DNA by size. Larger fragments do not move as fast as smaller fragments, and there is a nonlinear relationship between the size of the DNA fragments and the distance migrated. A DNA ladder, a sample with known fragment sizes, should always be run to identify the size of experimental bands.*

Agarose gel electrophoresis planning and results

Before you follow the agarose gel electrophoresis protocol for this lab, plan out what samples will be placed in each of your gel wells. Fill out the empty cells in the table below, noting their sample **contents** and the **DNA template**. Recall from the PCR amplification lab that you focused on two gene templates of known length: the 438 base pair (bp) 16S ribosomal RNA gene from *Wolbachia* and the 708 bp CO1 cytochrome c oxidase gene from arthropod mitochondria.

When we prepared the PCR reagent, we included a "green" dye in the master mix that includes a mix of two dyes (one blue and one yellow). The color makes it easy to view your sample as you load it in the gel well. The "green" dye then breaks into two visible bands during electrophoresis. The blue dye is composed of molecules similar to the 3–5 Kb DNA fragments in your arthropod and *Wolbachia* sample, and so gives you a sense where your DNA bands will be. In contrast, the yellow gel is very small (< 50 bp), and so travels ahead of your DNA bands. When this yellow band gets to the end of its lane, (and the blue is as far as possible from it so the DNA bands can be spread out), we know when to stop the electrophoresis.

Fill in the following table as you load the dye, so you know what each lane contains. Because we ran separate arthropod and *Wolbachia* thermal cycles while running PCR, we need to use two sets of wells. **Make sure that the arthropod samples are loaded in the top wells and the *Wolbachia* samples are loaded in the bottom wells.**

Make sure to include one well for each of the following, labelling each lane correctly. If you do not load a lane correctly, add a note in the table to explain the error and then load the next lane.

1. The 1 kilobase (kB) **ladder** standard serves as the reference for determining the size of DNA fragments.
2. **W+** control: The *Wolbachia*-positive control fruit fly from the prior lab.
3. **W-** negative control: The *Wolbachia*-negative control fruit fly from the prior lab.
4. *Wolbachia* DNA (**DNA+**) control: Purified DNA from fruit fly with *Wolbachia*
5. Water (**H_2O**) control: DNA-free water
6. The purified and amplified DNA samples from your team.

Once filled in, then provide a copy to your professor.

TURN IN: Hand in the following items to your professor for grading. Each team member must hand in their own copy of the pre-lab and post-lab, in their own words.

5. Pre-lab questions (due at start of lab).
6. Post-lab questions (due at start of next lab), including results table.
7. Digital image (photograph) of your labelled agarose gel.
8. Update your **Sample Collection forms** to note (a) the file names of your electrophoresis gel image (**Gel image – file name**) and (b) whether your insects tested for *Wolbachia* (***Wolbachia* (+) or (-)**).

8-well gel lane well key: Team #: _____ Section: _____ Date: _____

Team members: _____

Instructions:

1. Each 4-person team prepares a single gel.
2. Each well gets a **14 µl sample**: 2 µl of the arthropod sample(or W+ or W- control) + 2 µl of the *Wolbachia* sample (or W+ or W- control) + 10 µl loading gel). Only use **2 µl** for the DNA+ and H_20 controls (instead of 4 µl). The **ladder only uses 5 µl**.
3. As you add PCR product to each well, write the *sample ID* (e.g., FL3B), *control type* (W+, W-, DNA+, H_2O), or *ladder* placed in each well, **and** the PCR tube ID.
    - **Put the ladder in the first lane you use and the controls to its right.**
4. If you make a mistake with a lane, put "X" in it and add a note on the mistake (e.g., tip went through well, sample bled into adjacent well, etc.)
5. Put the arthropod and Wolbachia samples in the top lane and the control samples in the bottom lane.

Top lanes: Arthropod and *Wolbachia* samples

Lane#	1	2	3	4	5	6	7	8
Sample ID								
PCR tube ID								

Bottom lanes: Control samples

Lane#	1	2	3	4	5	6	7	8
Sample ID								
PCR tube ID								

Agarose Gel Electrophoresis Protocol

Class Materials

- ❑ Microwave
- ❑ LI-COR gel-documentation system

Materials per Group

- ❑ 70% ethanol
- ❑ Agarose powder
- ❑ Erlenmeyer flask
- ❑ Gloves
- ❑ SB buffer
- ❑ Kimwipes
- ❑ Electrophoresis chamber
- ❑ TBE buffer
- ❑ PCR tubes from prior lab
- ❑ Parafilm wax paper
- ❑ Sharpie marker
- ❑ Pipette (20–200)
- ❑ Pipette tips
- ❑ 5X Loading Buffer (with Orange G dye)
- ❑ 1 kB DNA ladder standard
- ❑ Waste cup for tips
- ❑ Waste cup for liquids

Add picture of casting tray

Prepare agarose gel

1. Remove all unnecessary items at your lab station. Clean all surfaces by wiping down with 70% ethanol.
2. Measure 2 g **agarose powder** and add it to a 500 ml flask.
3. Add 100 ml **SB Buffer** to the flask to produce a 2% agarose solution.
4. Melt the agarose in a microwave or hot water bath until the solution becomes clear. Look for "lenses" in the liquid by holding up against the light. If using a microwave, place a ball of Kimwipe tissue in the flask opening, or cover with film and poke a hole for venting, and heat the solution for several short intervals. **Watch carefully—do not let the solution boil over!**
5. Let the solution cool to about 50–55°C, swirling the flask occasionally so it cools evenly. *(See Note 8.1)*
6. Insert the sample well comb into the electrophoresis casting tray at the end position. The gates of the casting tray should be secured in the up position.
7. Pour approximately 40 ml of the hot agarose into your casting tray. *(See Note 8.2)*
8. Allow the agarose to solidify at room temperature for about 5 to 10 minutes. The gel will clear as it cools.
9. When set, carefully remove the comb by gently loosening it and then pulling it straight up. Lower the gates of the casting tray.

Add picture of set gel

Note 8.1: *The heated agarose solution should be cool enough that you are able to hold it on your hand for an extended time.*

Note 8.2: *Fill the gel in the tray so that only the teeth of the comb are immersed under the gel, but the base of the comb is just above the gel.*

Add picture of electrophoresis chamber

Prepare the gel chamber

10. Transfer the gel tray to the platform in the electrophoresis chamber, such that the sample wells are closest to the black (negative) electrode.
11. Fill the electrophoresis chamber with the **TBE buffer solution** such that the gel is covered with a 2.5 mm layer of buffer.

Add another picture?

Agarose Gel Electrophoresis Protocol

Load the samples

12. Collect your two sets of eight PCR tubes from the PCR Amplification Lab and place them in your PCR strip holder. Put the arthropod strip **above** the *Wolbachia* strip.
    - Make sure that the strip is oriented from left to right in the same order as the sample ID table in the PCR Lab (and in the order of the gel lane key).
13. Collect an 8-inch-long strip of Parafilm wax paper and lay it on your lab bench **on top of the sample ID key so you can keep track of what goes in each drop**. Use a Sharpie marker to write the numbers 1–8, spaced approximately 1-inch apart. You will mix your gel samples on this Parafilm. (*See Note 8.3)* Next to each number, **write the name of each sample (e.g., FL3B) or control (e.g., W+, W-, DNA+, H_20)**.
14. Pipet a **10 µl drop of loading buffer dye** near each of the numbers. (You should have 8 drops.) You can use the same pipette tip for this step. (See Note 8.4.)
15. Using a new pipette tip for each PCR tube sample, add **2 µl of the arthropod** PCR reaction tube sample **and 2 µl of the matching *Wolbachia*** PCR sample to the appropriate **drops 1–4** of loading buffer. Then mix well ('plunge') by gently pipetting up and down several times until the color of the liquid in each drop is homogenous.
16. Repeat for drops 5–8 using the four control samples, but adding **2 µl of the DNA+ and H_20 controls**.

Storage

17. Store the remaining PCR tube solutions in the freezer in case you need to re-run this protocol again. If a sample turns out to have *Wolbachia* DNA present, you will submit the sample to have its DNA sequenced for a later lab.

Load the gel

18. Review the contents of each drop. As you load each sample (including controls) into the gel wells, **record each sample and PCR ID in the key on the prior page**.
    - If you make a mistake as you load a well, add a note to the sheet (e.g., tip went through well, sample bled into adjacent well, etc.) so you can correctly interpret the gel later. Then add your sample to the next well.
19. Moving from left to right and using a new pipette tip for each drop, add **14 µl of each sample + Loading Buffer mixture** into their corresponding well in the gel. Put the **4 samples in the top lanes and the 4 controls to the bottom lanes**.
20. Carefully pipette **5 µl of the 1 kB DNA ladder standard** into the rightmost well (#5) and **write "ladder" on the table**.
    - We use only 5 µl so that the DNA bands will separate more efficiently.

Running the gel

21. Make sure the voltage knob on the electrophoresis unit's power pack is turned down to zero.
22. Place the electrophoresis unit lid into position and attach the electrophoresis leads (black/negative and red/positive wires) to the power pack. **Be sure you plug the correct wires into the correct holes.** *(See Note 8.5.)*

Note 8.3: *This is the most important step. Be careful to keep each drop separate and well spaced from each other so that you do not contaminate samples. Always use a new pipette tip with each sample and solution! You can use the tip for each PCR tube to mix this solution with the drop of buffer.*

Note 8.4: *The loading buffer is viscous to make it easier to load your samples into the wells.*

Note 8.5: *The DNA will move from the black (negative) end where the wells are, to the red (positive) end. Ask the instructor or a TA if you are not sure.*

Note 8.6: *Check to make sure the current is running through the buffer by looking for bubbles forming on each electrode. After a few minutes, confirm that the current is running in the correct direction by observing the movement of loading dye.*

Add picture of Parafilm mixing

Add picture of adding samples to wells

Add picture of correct wire configuration

Add another picture?

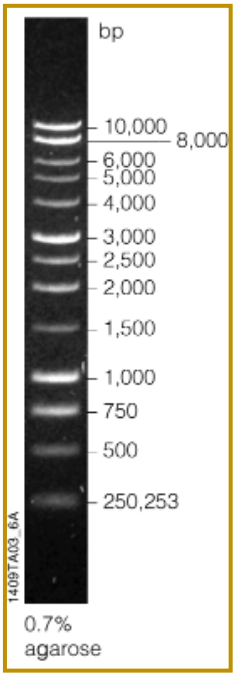
23. Turn on the power pack and adjust the voltage knob to 100 volts. *(See Note 8.6.)*

Agarose Gel Electrophoresis Protocol

24. Allow the samples to electrophorese for about 50 minutes, until the yellow dye in the DNA sample solutions has migrated most of the way (5–25 mm) from the end of the gel facing the positive (red) electrode, and the blue dye is in the middle of the gel lane.
25. Turn the voltage down to zero, shut off the power pack, and unplug it. Disconnect all wires.

Viewing the gel

26. Carefully remove the casting tray containing your gel. Slide your gel into a plastic staining tray labeled with your lab section time and group number. Rinse the gel once with distilled or reverse osmosis (RO) water.
27. Bring the gel to the LI-COR gel-documentation system. Your instructor will photograph your gel and send you a PDF file of the image.
28. Clean your lab table by wiping down with 70% ethanol.
29. When received, print your gel image. Label the lanes by the sample or control contents in the table above. Label the DNA ladder standard bands according to the picture of a 1kb ladder at right. Note the different intensities of some of the bands in the ladder diagram. These bright bands will help you find your spot on the ladder in your gel lane. Not all bands may be visible (smaller ones are dimmer) or separated completely (larger ones).
30. Take a digital image (photograph) of your labeled gel image.



Name:_____ Lab Section:_____

GEL ELECTROPHORESIS

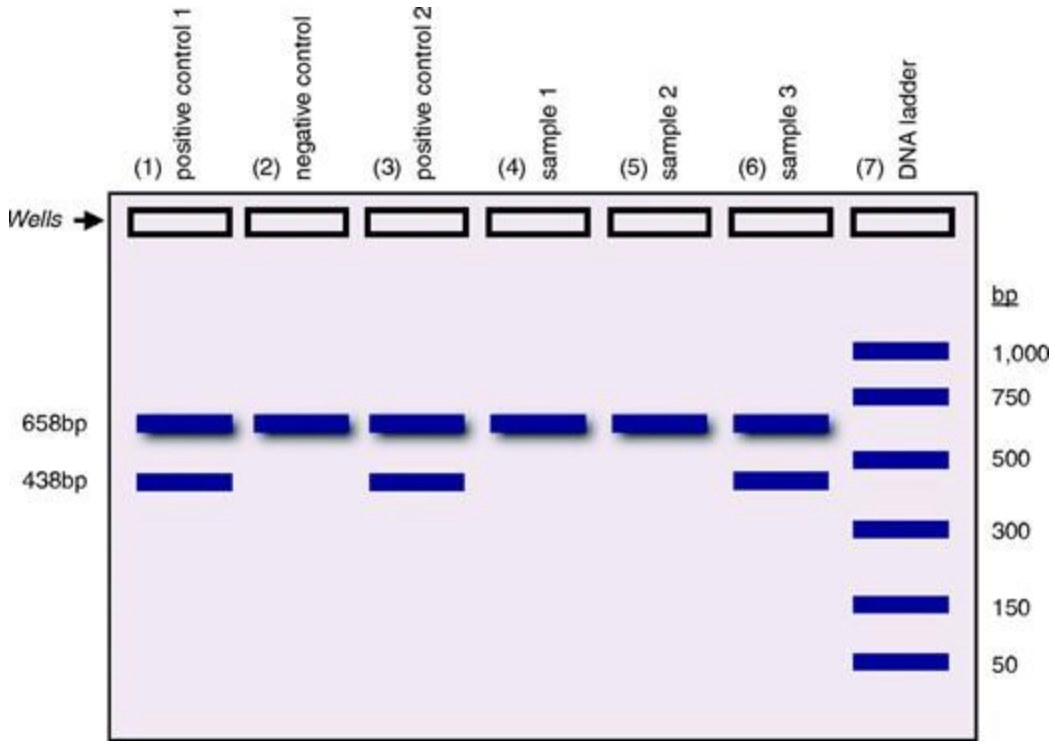
Pre-Lab Questions (due at start of lab)

Read through the lab introduction and entire lab protocol to answer the questions below.

7. Does DNA move toward the positive or negative charge in gel electrophoresis? Why?

8. What is a 1 kB DNA ladder standard, and why is it used in gel electrophoresis?

9. Observe the image below of a hypothetical agarose gel after running and visualization (paying close attention to the positive and control groups). On the next page, fill in the table based on your interpretation of this gel.



Referring to the gel on the prior page, fill in the table of results below, noting whether samples 1, 2 and 3 indicate the presence (or absence) of animal CO1 genes and *Wolbachia* 16S ribosomal RNA gene.

Lane #	Contents	CO1 (+/-)	*Wolbachia* (+/-)
4	Insect sample #1		
5	Insect sample #2		
6	Insect sample #3		

10. Explain how you inferred these conclusions.

Name:_____ Lab Section:_____

GEL ELECTROPHORESIS

Post-Lab Questions (due at start of next lab)

Read through the lab introduction and entire lab protocol to answer the questions below.

1. Interpret the results of your gel electrophoresis. Use the intensity of bands to identify how abundant the DNA fragments are in each sample, including the controls. Did you get bands showing arthropod DNA? Were your insects infected with *Wolbachia*? How abundant were each type of DNA? Explain how you made these conclusions, referring to your gel electrophoresis results (including the results of the various control groups).

2. Based on the classwide results, which insect order is most commonly infected with *Wolbachia*? Which is least? Explain how you concluded this.

(continued on other side)

3. Based on the classwide results (see table at end of post-lab), which insect **orders** have the greatest frequency of *Wolbachia* infection? Which have the lowest?

4. Based on the class-wide results (see table at end of post-lab), which insect **habitats** have the greatest frequency of *Wolbachia* infection? Which have the lowest? (You can skip this question in Spring semester if information on sampled habitat is not available.)

5. Thinking back across the DNA extraction, amplification, and today's confirmation labs, were there any errors that occurred? What could you have done differently to improve the reliability of the results. What does this teach you about doing science?

Share on D2L your results for your insect samples with the rest of the class so you can observe which insects included endosymbiotic *Wolbachia* bacteria.

Group initials	Insect order	Habitat	COI (+/-)	*Wolbachia* (+/-)
			-	-